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Effect of pioglitazone on body composition and energy expenditure: a randomized controlled trial

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Abstract

Background: Several clinical studies have demonstrated that body weight increases after treatment with thiazolidinediones (TZDs). Prior studies have demonstrated an increase in insulin-stimulated lipid storage in adipose tissue. Some, but not all, studies demonstrate reductions in visceral adipose tissue. Changes in body weight are the result of changes in energy intake, energy expenditure, or both.
Objectives: Based on these findings, the primary aim of this study was to evaluate the effect of TZDs on visceral, subcutaneous, and total body fat. Secondary aims were to determine the effects of pioglitazone on (*a*) energy expenditure, (*b*) hunger and satiety, (*c*) blood lipids, and (*d*) the role of insulinemia/sulfonylurea usage on weight gain in patients with type 2 diabetes.
Subjects and Methods: We performed a randomized, double-blind, placebo-controlled trial in 48 men and women with type 2 diabetes who had not previously received treatment with TZDs. Patients were treated for 24 weeks with 45 mg/d of pioglitazone or a matching placebo. Body composition was measured by dual-energy x-ray absorptiometry. Visceral and subcutaneous fat were measured by computed tomography. Resting metabolic rate and thermogenic response to a test meal were measured by indirect calorimetry before and after a standardized meal. Hunger and satiety were measured with visual analog scales before and after the same test meal. Blood was collected for the measurement of fasting glucose and insulin levels, hemoglobin A_{1c} levels, and lipid content.
Results: Pioglitazone treatment resulted in a decrease in hemoglobin A_{1c} level by 0.96 ± 1.1% vs 0.11 ± 0.8% in the placebo group ($P < .005$). Body weight and fat increased steadily in the patients treated with pioglitazone during the 6 months of the study (+3.9 ± 3.1 kg at 6 months in pioglitazone-treated patients vs −0.8 ± 3.4 kg in the placebo-treated patients). Subcutaneous fat in the trunk, arms, and legs were all increased in the pioglitazone-treated group. Visceral fat did not change significantly in either group. Neither resting metabolic rate nor the thermogenic responses to a meal were altered by pioglitazone. Subjective measures of hunger (visual analog scale) did not change with pioglitazone treatment. Triglycerides fell in the pioglitazone-treated group (−58.5 ± 124 mg/dL, $P < .003$). Neither the prior use of sulfonylureas nor the level of insulinemia before treatment was a predictor of weight or fat change.
Conclusion: Pioglitazone increased subcutaneous body fat, but not visceral fat. There was no measurable effect on energy expenditure or hunger/satiety. In contrast to the placebo-treated patient with diabetes, weight gain occurs in the face of falling hemoglobin A_{1c} and triglyceride levels.

1. Introduction

Pioglitazone is a member of the thiazolidinedione (TZD) class of drugs and is a potent agonist for the peroxisome proliferator-activated receptor-γ (PPAR-γ). In the clinical setting, the drug enhances the sensitivity to insulin, providing its indication for use in states of insulin resistance such as diabetes and the polycystic ovary syndrome.

In cell cultures, PPAR-γ activators are involved in the early stages of fat cell differentiation, leading cultures of preadipocytes to begin differentiating into mature fat cells [1-3]. In rodents, this differentiation of fat cells is manifested as an increase in the number of small adipocytes [4,5].

Several investigators have demonstrated that troglitazone increases subcutaneous abdominal adipose tissue (SAT) and may decrease visceral adipose tissue (VAT) when given to humans with diabetes [6-8]. In contrast, when troglitazone was given to patients receiving sulfonylureas, subcutaneous fat increased, but visceral fat did not change [7]. This

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suggests that the drug has specific effects on SAT, consistent with regional differences in PPAR-γ gene expression [9]. It is possible that the concomitant use of sulfonylureas along with TZDs stimulates β-cell insulin secretion and maintains lipid stores in VAT, preventing loss of VAT. Conversely, for individuals who are not taking sulfonylureas, insulin levels fall with the improvement in insulin sensitivity, allowing the release of lipid from the visceral depot.

These results suggest, but do not prove, that the modest weight gain associated with TZDs is the result of differentiation of quiescent preadipocytes into insulin sensitive, lipid-storing small fat cells [10,11]. If this sequence of events is operative in vivo, and energy expenditure remained unchanged, any increase in body fat would, by necessity, be due to increases in energy intake. We hypothesized that pioglitazone significantly increases subcutaneous body fat, decreases visceral fat, but does not affect energy expenditure in obese diabetic patients, and that this change in body fat might depend on basal insulinemia or the use of sulfonylurea.

The primary aim of this study was to evaluate the effect of TZDs on visceral, subcutaneous, and total body fat. Secondary aims were to determine the effects of pioglitazone on (*a*) energy expenditure, (*b*) hunger and satiety, (*c*) blood lipids, and (*d*) the role of insulinemia/sulfonylurea usage on weight gain in patients with type 2 diabetes.

2. Methods and procedures

2.1. Subjects

After reading and signing an informed consent document, 48 men and women, aged 35 to 75 years, with type 2 diabetes as defined by a fasting plasma glucose of 126 mg/dL or higher at entry or fasting plasma glucose of more than 115 mg/dL and a 2-hour oral glucose tolerance test glucose of 200 mg/dL or higher, were eligible to participate in a protocol approved by the local Institutional Review Board. Fasting plasma glucose at entry had to be 200 mg/dL or less. For women, use of adequate contraceptive control was required. This could include oral contraceptives, hysterectomy, tubal ligation, or postmenopausal status, as defined by more than 6 months without a menstrual cycle and follicle stimulating hormone level of 40 mIU/mL or higher. Patients could be treated with diet, metformin, or sulfonylurea; the latter two were continued as necessary through the study. Patients were excluded if they had significant renal, cardiac, liver, lung, or neurological disease, although controlled hypertension was acceptable if baseline blood pressure was less than 140/90 mm Hg on medications. Patients with prior use of TZDs (troglitazone, rosiglitazone, pioglitazone), β-blockers, current pregnancy, smokers, alcohol or other drug abuse, or unwilling to abstain from caffeine for 48 hours and alcohol for 24 hours before metabolic rate measurements were also excluded. If liver function tests at baseline (aspartate transaminase, alanine transaminase, γ glutamyl transpeptidase, or alkaline phosphatase) were greater than 2.5 times the upper limit of normal, the subjects were not enrolled. Metal objects that would interfere with the measurement of visceral fat with computed tomography (CT) such as implanted rods or surgical clips prevented patients from participating. In addition, if patients were taking drugs known to affect lipid metabolism, energy metabolism, or body weight, such as orlistat, sibutramine, ephedrine, phenylpropanolamine (Dexatrim), or corticosteroids, they were rejected.

2.2. Methods

Patients received the usual care for their diabetes with instruction on a healthy diet for diabetic patients by a dietitian. Diet instruction was based on published American Diabetes Association guidelines and included instruction on caloric counting, portion control, and reduction in saturated fat. Energy (caloric) levels were based on the World Health Organization (WHO) calculations and on activity level of 1.3 times the basal energy requirements [12]. Blood glucose level monitoring was encouraged. Subjects were enrolled into a 24-week double-blind, placebo-controlled, randomized, parallel arm study with 2 arms: pioglitazone and placebo. The hemoglobin A_{1c} target was less than or equal to 7.0%. Pioglitazone and matching placebos were prepared by Takeda Pharmaceuticals (Lincolnshire, Ill) and were given as a single daily dosage of 30 mg/d, or placebo, each morning. If, after 8 weeks, the hemoglobin A_{1c} level was greater than 7.0% or the fasting plasma glucose level was greater than or equal to 100 mg/dL, the dosage of pioglitazone (or matching placebo) was increased to 45 mg/d, which occurred in all but one participant. If individuals had an increase in the hemoglobin A_{1c} level of more than 12% or an increase in the fasting plasma glucose level of more than 240 mg/dL, they were to be treated with sulfonylureas or insulin, but this was not necessary in this study. Subjects on sulfonylureas or metformin who experienced hypoglycemia had the dose of these medications reduced or the medication discontinued. Patients visited the clinic weekly for 4 weeks and each month thereafter. During these visits, blood pressure was measured and a blood sample was collected for determination of hemoglobin A_{1c}, fasting glucose (week 8 and end of study), serum lipids, and liver function tests. QUICKI, an index of insulin resistance previously validated in diabetic subjects, was calculated as described by Katz et al [13] and Chen et al [14].

2.3. Subjective measures of hunger (visual analog scale) and fullness

A series of questions was administered using a laptop computer with proprietary Pennington Biomedical Research Center software after an overnight fast at baseline and hourly during the measurement of the thermogenic effect of food (TEF) at baseline and at 6 months of therapy with pioglitazone. Subjects were asked to place a mark, reflecting their current evaluation of the question, on a line that was

anchored at each end indicating the extreme of "happiest I have ever been" or "not happy at all," etc. The questions were as follows:

1. How happy do you feel at this moment?
2. How hungry do you feel at this moment?
3. How calm do you feel at this moment?
4. How thirsty do you feel at this moment?
5. How full does your stomach feel at this moment?
6. How much food do you think you could eat at this moment?
7. How content do you feel at this moment?
8. How anxious do you feel at this moment?
9. How strong is your desire to eat at this moment?

2.4. Body composition

Total and regional body fat (arm, leg, and trunk) were determined by dual-energy x-ray absorptiometry (DEXA) with the Hologic QDR4500 (Waltham, Mass) at baseline, week 12, and week 24. Visceral and abdominal subcutaneous fat were measured using a multislice CT scan technique on a GE CT scanner (Milwaukee, Wis) at baseline, week 12, and week 24. Eight images were obtained: L4-5, 2 images at 5 and 10 cm below L4-5, and 5 images every 5 cm above L4-5. Subcutaneous and visceral fat were quantitated using established image analysis procedures [15]. Body weight was measured at each visit on a calibrated electronic scale to the nearest 0.1 kg. Anthropometric measurements included height, weight, waist, and thigh circumference. Waist circumference was measured at the level of the umbilicus. Thigh circumference was measured at the gluteal-thigh crease.

2.5. Energy expenditure

Resting metabolic rate (RMR) and the change in metabolic rate after a meal were determined over a 7-hour period both at baseline and at the 24-week time point using a ventilated hood system (Deltatrac II Metabolic Monitor, Datex-Ohmeda, Helsinki, Finland). Calculations of O_2 consumption (VO_2) and CO_2 production (VCO_2) were made from continuous measurement of CO_2 and O_2 concentrations in inspired and expired air diluted in a constant airflow (~40 L/min) generated by the analyzer. The device was calibrated every hour against a reference gas containing ~5% CO_2 and 95% O_2.

On the test day the participant arrived at the metabolic unit at 7:00 AM and after a 30-minute supine rest, measurement of fasting energy metabolism was started for a period of 30 minutes, followed by the ingestion of the test meal. The test meal contained energy equivalent to 30% of basal metabolic rate, as calculated using the WHO equations, with 30 g of white bread, 5 g of butter, 2 eggs, 200 mL of 2% milk, 10 mL of 10% cream, 200 mL orange juice, and 10 g of sugar, representing 38.5 g carbohydrate, 18.1 g protein, and 18.4 g fat [16]. To obtain the desired energy (caloric) intake, the amounts of each food item were multiplied by the required factor. Upon completion of the test meal, postprandial metabolism was measured for 30 minutes at hourly intervals for a total of 6 hours. During the 30 minutes between measurements the subjects remained resting. Subjects voided before beginning the test, and the urine produced during the test was collected to measure urinary nitrogen excretion, which was used to correct oxygen consumption for protein oxidation. VO_2 and VCO_2 were converted to carbohydrate, fat oxidations (FOX), and protein oxidations (POX) using the equations of Elia and Livesey [17]. Hunger and satiety were measured every 60 minutes throughout the test for the TEF using a digital visual analog scale (VAS) on a laptop computer.

2.6. Statistical analysis

All data are presented as mean ± SEM (or SD as indicated) for all response variables with multiple measurements over time. Repeated measures ANOVA was used with the time (weeks or months) as a repeated factor. Treatment, time, and the treatment by time interaction were the main fixed effects in each model. For the variables that were measured only before treatment and after treatment at the end of 24 weeks, the change from baseline was analyzed with the baseline as a covariate using a simple linear model. In all cases, unless specifically indicated otherwise, the change from baseline calculated as difference at a particular time point (week) and baseline was used as the response variable, and the baseline was used as a covariate in each model. In the analysis of triglycerides and insulin, the log-transformed response was used. Each of the 9 VAS questions, administered before a meal at baseline, at 12 weeks, and at 24 weeks, was analyzed separately using a repeated measures design and also as a multivariate response using a Hotelling-Lawley-Pillai-Samson statistic. Multiple-comparison P values were adjusted using the Tukey-Kramer method. All analyses were performed using SAS Version 8.2 software (SAS Inc, Cary, NC).

3. Results

3.1. Subjects

The baseline characteristics for the 21 patients completing the study are summarized in Table 1 separated by group and gender. Nine subjects in the placebo arm and 11 subjects in the pioglitazone arm were taking sulfonylurea at study entry. Five subjects in the placebo arm and 6 subjects in the pioglitazone arm identified themselves as non-White.

Of the 48 subjects, 42 completed the trial with equal dropouts in each arm. The characteristics of the 48 subjects at baseline were similar to those who completed the trial. For example, the body mass index (BMI) of the placebo group (n = 24) at entry was 31.8 ± 4.8 kg/m^2 vs 32.4 ± 5.4 for the subjects assigned to receive pioglitazone (also n = 24). The mean body weight of the placebo group (n = 24) at entry was 92.1 ± 14.6 vs 93.5 ± 18.5 kg/m^2 for the subjects assigned to receive pioglitazone (also n = 24).

Table 1
Baseline characteristics of the two treatment groups

Variable	Placebo		Pioglitazone		*P*
No. of subjects (M/F)	21 (10/11)		21 (9/12)		
Race (White/non-White)	16/5		15/6		
Taking sulfonylurea (yes/no)	9/12		11/10		
Age (y)	53.1 ± 9.3		56.2 ± 9.7		.2050
Anthropometry					
Height (cm)	169.6 ± 10.0		170.7 ± 10.9		.70339
Weight (kg)	91.5 ± 14.9		93.5 ± 19.6		.7154
BMI (kg/m^2)	31.9 ± 5.0		32.1 ± 5.6		.8968
	Female	Male	Female	Male	
Waist (cm)	100.7 ± 14.9	101.7 ± 24.5	100.9 ± 17.1	109.2 ± 17.5	.5694
Thigh	64.8 ± 17.1	61.3 ± 5.1	67.8 ± 13.2	61.6 ± 8.2	.5847
Waist/thigh ratio	1.6 ± 0.4	1.7 ± 0.4	1.5 ± 0.3	1.8 ± 0.1	.8166
DEXA data					
Body fat (kg)	34.5 ± 8.2	28.3 ± 7.5	36.3 ± 8.4	29.0 ± 15.5	.6131
Body fat (%)	40.1 ± 3.6	28.5 ± 4.3	41.6 ± 3.5	27.2 ± 7.5	.7331
Lean body mass (kg)	50.5 ± 5.9	69.7 ± 6.8	50.2 ± 7.0	72.7 ± 8.7	.9677
Trunk fat (kg)	17.7 ± 3.5	16.7 ± 5.5	18.3 ± 5.4	16.5 ± 9.0	.8549
Leg fat (kg)	11.6 ± 4.4	7.6 ± 1.4	12.4 ± 2.5	8.3 ± 4.7	.4585
Arm fat (kg)	4.3 ± 1.1	2.9 ± 0.8	4.7 ± 1.4	3.0 ± 1.9	.4767
Computed tomography					
VAT (kg)	4.9 ± 1.6	7.0 ± 1.7	4.9 ± 2.2	6.8 ± 1.9	.7617
SAT (kg)	11.0 ± 3.4	9.4 ± 3.9	12.9 ± 3.2	7.1 ± 2.7	.7422
SAT (cm^2)	335 ± 135	295 ± 111	347 ± 103	280 ± 192	.9505

Data are mean ± SD. *P* values are presented for the comparison of each baseline characteristic across treatment by ANOVA.

The 42 completers were well matched on anthropometry, body composition determined by DEXA, and CT of abdominal fat. The average age in the 2 groups was 53 and 56 years. Height, weight, BMI, and the circumferences of the waist and thigh were similar and did not differ between groups. Women had more fat than a less lean body mass man, but there were no differences between treatment groups for women or men. Leg and arm fat were significantly larger in women than in men, but trunk fat by CT was similar between men and women. Visceral fat was significantly smaller by about 30% in women than in men. In contrast, subcutaneous fat was larger in women than in men.

Table 2
Baseline values and changes from baseline in glucose, hemoglobin A_{1c}, and serum lipids

	Placebo			Pioglitazone			*P*		
	Baseline	12 wk	24 wk	Baseline	12 wk	24 wk	Treatment	Time	Interaction
Hemoglobin A_{1c} (%)	6.46 ± 0.72	–	−0.11 ±0.79	6.88 ± 1.35	–	−0.96 ± 1.11	.0054*	.0398*	.0256*
Glucose (mg/dL)	151.96 ± 36.66	−6.41 ± 40.25	2.40 ± 33.65	157.83 ± 43.21	−27.05 ± 31.47	−25.10 ± 25.69	.0031*	.2298	.0936
Insulin (mIU/mL)	15.01 ± 9.66	−3.20 ± 6.69	−1.95 ± 4.79	14.38 ± 9.06	−5.94 ± 6.75	−3.89 ± 8.10	.0021*	.0668	.1387
QUICKI (AU)	0.31 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.31 ± 0.03	0.03 ± 0.02	0.02 ± 0.02	<.0001*	.451	.273
Triglycerides (mg/dL)	221.96 ± 141.66	−18.23 ± 77.35	−2.36 ± 59.87	205.79 ± 182.61	−54.18 ± 134.85	−58.52 ± 123.26	.0035*	.7951	.1617
HDL-C (mg/dL)	46.90 ± 10.05	2.34 ± 4.25	1.44 ± 3.77	47.45 ± 14.34	6.68 ± 6.10	7.77 ± 5.22	.0003*	<.0001*	<.0001*
LDL-C (mg/dL)	103.51 ± 23.22	1.65 ± 14.21	6.78 ± 18.97	107.40 ± 32.97	10.81 ± 37.71	18.29 ± 26.86	.3538	.0017*	.0226*
Total cholesterol (mg/dL)	191.04 ± 29.35	3.36 ± 20.12	8.19 ± 20.88	190.54 ± 30.96	11.50 ± 38.82	19.57 ± 26.14	.3822	.0006*	.0325*

Data are mean ± SD. AU indicates arbitrary unit; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Forty-two subjects (21 in each treatment group) were included in this analysis.

* $P \leq .05$.

Overall, pioglitazone treatment was well tolerated in this population. Three patients noted "increased hunger" as an adverse event, whereas none of the placebo-treated patients reported this as an adverse event ($\chi^2 = 3.2$; $P = .234$). One patient in the placebo group and 2 patients in the pioglitazone-treated group developed peripheral pitting edema. One of the patients treated with pioglitazone developed pulmonary edema, confirmed by chest x-ray that required diuretic therapy. This occurred in the absence of cardiac disease and resolved after dose reduction.

The baseline laboratory data and changes from baseline are summarized in Table 2. Here again the groups were well matched at baseline. At 24 weeks, there was a significant decrease of nearly 1% in hemoglobin A_{1c} level in the patients treated with pioglitazone as compared with baseline (6.88%-5.92%, $P < .001$). Glucosuria was present in only 2 subjects: 1 in the placebo group and 1 in the pioglitazone treatment group. In both subjects, glucosuria was absent at the 3- and 6-month time points. Fasting insulin concentration decreased significantly more in the pioglitazone-treated group than in the placebo group (−3.2 μU/mL at 12 weeks, $P < .0001$), and this effect was evident during all measured weeks (Fig. 1A).

There was also a significant fall in fasting triglyceride concentration ($P < .01$ for all measured weeks) in those patients treated with pioglitazone (Fig. 1B). At 24 weeks, there was a significant increase in both fasting high-density lipoprotein cholesterol levels and fasting low-density lipoprotein cholesterol levels (for both $P < .0001$).

Pioglitazone treatment resulted in a significant decrease in insulin resistance as measured by the QUICKI index, which was attenuated over time (treatment effect, ie, pioglitazone vs placebo, at each of the following periods: week 2, $P = .0006$; week 4, $P = .0002$; week 8, $P = .02$; week 12, $P < .0001$; week 16, $P = .06$; week 20, $P = .005$; and week 24, $P = .51$).

3.2. Body weight

The change in body weight and the 12- and 24-week data presented in Table 3 are graphed in Fig. 1C. Within the first 2 months, the body weight was significantly higher in the patients treated with pioglitazone (0.9 ± 2.4 kg, $P = .04$). Body weight continued to rise throughout the trial in the pioglitazone-treated group and had not reached a plateau by the end of the study (+3.88 ± 3.11 kg). In contrast, body weight decreased, although not significantly, by 0.79 ± 3.36 kg in the placebo group.

The participants treated with pioglitazone were separated into those who received sulfonylurea and those who did not (Table 4). Please note that this was not a treatment assignment, per se; this category indicates whether the subject was taking sulfonylurea at study entry. Based on the information reviewed in the Introduction, the question is whether the use of sulfonylurea influences the weight and fat gain seen with pioglitazone; thus, the placebo group was not included in this analysis. In contrast to the predicted result, the pioglitazone-treated patients and the two sulfonylurea subgroups had similar initial weights, but those receiving a sulfonylurea drug gained slightly, but not significantly, less weight than those who did not take sulfonylureas at study entry (3.50 ± 3.10 vs 4.29 ± 3.23 kg; Table 4).

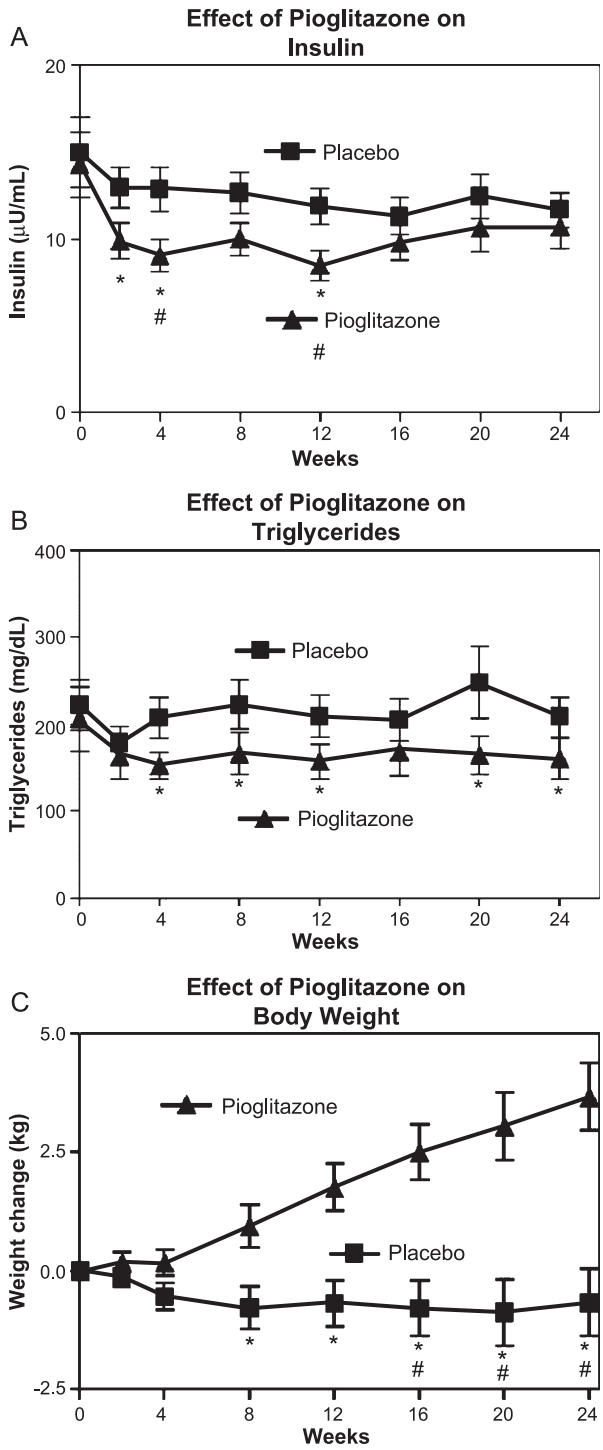

Fig. 1. Effect of pioglitazone on change in insulin, triglycerides, and body weight. Values are plotted as change from baseline (mean ± SEM). Asterisk indicates $P < .05$; number sign, $P < .05$ after Tukey-Kramer adjustment for multiple comparisons. Forty-two subjects (21 in each treatment group) were included in this analysis.

Table 3
Baseline values and change from baseline in the intent-to-treat population for body weight, DEXA measures, VAT, and SAT

	Placebo			Pioglitazone			*P*		
	Baseline	12 wk	24 wk	Baseline	12 wk	24 wk	Treatment	Time	Interaction
Weight (kg)	92.13 ± 14.65	−0.71 ± 2.19	−0.79 ± 3.36	93.48 ± 18.48	1.68 ± 2.46	3.88 ± 3.11	.0004*	.0262*	.0107*
Waist (cm)	99.96 ± 20.13	3.84 ± 24.23	3.13 ± 25.67	104.96 ± 16.45	2.91 ± 9.38	4.01 ± 9.87	.8687	.5991	.1233
Thigh (cm)	64.66 ± 14.25	−0.55 ± 14.47	−0.29 ± 15.45	65.33 ± 10.77	−0.57 ± 8.36	0.86 ± 7.91	.0818	.0098*	<.0001*
DEXA fat (kg)	31.21 ± 7.96	−0.62 ± 1.56	−0.47 ± 2.54	33.43 ± 11.65	1.13 ± 1.50	3.55 ± 2.52	<.0001*	.0003*	.0007*
DEXA fat (%)	34.06 ± 6.93	−0.26 ± 1.20	−0.16 ± 1.53	35.77 ± 8.88	0.47 ± 0.99	2.05 ± 1.43	<.0001*	.0005*	.0012*
DEXA trunk fat (kg)	17.07 ± 4.33	−0.31 ± 1.01	−0.07 ± 1.25	17.41 ± 6.61	0.17 ± 0.85	1.58 ± 1.87	.0025*	.0008*	.0106*
DEXA leg fat (kg)	9.49 ± 3.70	−0.25 ± 0.74	−0.28 ± 1.05	10.91 ± 4.02	0.83 ± 0.82	1.66 ± 1.18	<.0001*	.0038*	.0009*
DEXA arm fat (kg)	3.66 ± 1.11	−0.07 ± 0.33	−0.14 ± 0.46	4.08 ± 1.76	0.10 ± 0.29	0.29 ± 0.36	.0062*	.0542	.0058*
Lean (kg)	60.57 ± 11.66	−0.22 ± 1.37	−0.36 ± 1.43	59.57 ± 13.15	0.60 ± 1.38	0.14 ± 1.72	.0972	.2587	.4773
VAT (kg)	5.97 ± 1.90	−0.14 ± 0.40	−0.09 ± 0.58	5.46 ± 2.28	−0.23 ± 0.33	−0.12 ± 0.34	.5860	.0432*	.7065
SAT (kg)	9.98 ± 3.44	−0.25 ± 0.70	−0.27 ± 0.99	10.91 ± 4.19	0.57 ± 0.77	1.10 ± 1.02	.0005*	.0050*	.0166*
SAT (cm^2)	304.21 ± 120.12	−6.92 ± 30.76	−2.45 ± 29.26	326.60 ± 143.21	22.87 ± 31.13	44.18 ± 38.73	.0002*	<.0001*	.0306*
VAT (cm^2)	258.36 ± 85.38	−7.77 ± 29.66	−0.85 ± 36.72	230.46 ± 99.65	−10.39 ± 24.40	−10.18 ± 24.53	.423	.19	.343
Liver/ spleen (AU)	1.00 ± 0.23	0.03 ± 0.17	0.03 ± 0.23	1.12 ± 0.22	0.06 ± 0.12	0.06 ± 0.12	.1050	.8597	.8513

Data are mean ± SD. VAT and SAT (cm^2) measured at L4-5. Forty-two subjects (21 in each treatment group) were included in this analysis.
* $P \leq .05$.

We next tested whether hyperinsulinemia was related to the change in body weight in those subjects treated with pioglitazone by correlating the change in body weight with the fasting insulin level at screening. There was no significant correlation between these 2 variables (results not shown; $P > .05$).

3.3. Body composition

Baseline values and changes from baseline in body composition are shown in Table 3. Waist circumference increased in both groups, but the increase was not significantly different between the two groups. Thigh circumference, in contrast, increased in those treated with pioglitazone but decreased in the placebo-treated group.

Using DEXA to measure total body fat mass and composition, we found that essentially all of the weight gain in the pioglitazone-treated patients at 24 weeks was fat (3.55 ± 2.52 kg, $P < .0001$).

Abdominal subcutaneous fat, measured by multislice scanning (and traditional single-slice scanning) showed significant increases with pioglitazone treatment, but no change in the placebo-treated group. Multislice measurement of VAT mass showed a small but significant decrease from baseline to week 12 in the group treated with pioglitazone ($P = .0058$), but not in the placebo-treated group ($P = .075$). However, there was no significant difference in VAT mass between the two groups at 12 weeks. At 24 weeks, the VAT mass for both placebo- and pioglitazone-treated patients had returned toward baseline and were not different from baseline or from each other.

Table 4 shows data after stratifying the groups into those who received sulfonylurea treatment and those who did not receive these drugs. Using a 2-way ANOVA, we analyzed the changes in body weight, fat measured by DEXA, and visceral fat and subcutaneous fat measured by CT in subjects taking pioglitazone. We found that the changes in

Table 4
Effect of sulfonylurea treatment on the change in VAT during treatment with pioglitazone

Variable	SU	Pioglitazone			*P*		
		Baseline	12 wk	24 wk	Treatment	Time	Interaction
Weight (kg)	N	95.32 ± 23.05	1.66 ± 2.29	4.35 ± 3.07	.7481	.0002*	.3112
	Y	91.29 ± 11.75	1.70 ± 2.80	3.35 ± 3.22			
DEXA fat (kg)	N	34.01 ± 12.56	1.09 ± 1.65	4.09 ± 2.61	.4665	<.0001*	.2355
	Y	32.74 ± 11.02	1.17 ± 1.38	2.94 ± 2.39			
VAT (kg)	N	5.37 ± 2.33	−0.13 ± 0.32	−0.16 ± 0.39	.2690	.1009	.3140
	Y	5.55 ± 2.32	−0.34 ± 0.31	−0.14 ± 0.26			
SAT (kg)	N	10.90 ± 3.90	0.44 ± 0.80	0.89 ± 1.05	.3400	.0015*	.6384
	Y	10.91 ± 4.67	0.71 ± 0.75	1.28 ± 1.00			
SAT (cm^2)	N	345.30 ± 154.73	19.69 ± 37.89	44.87 ± 46.27	.4880	.0004*	.4241
	Y	304.49 ± 132.08	26.68 ± 21.82	43.40 ± 30.85			

Data are mean ± SD. SU indicates sulfonylurea usage at entry to study; N, not taking sulfonylurea; and Y, taking sulfonylurea. n = 21 completers.
* $P \leq .05$.

body weight, in fat measured by DEXA, and in visceral fat and subcutaneous fat measured by CT were not different in the patients treated with pioglitazone taking sulfonylurea compared with those patients treated with pioglitazone receiving no sulfonylurea at study entry.

3.4. Hepatic lipid content

Next, we asked whether these changes in body fat adversely affected "ectopic fat" in the liver. The liver/spleen ratio is a measure of ectopic fat in the liver, with a high value indicative of a low lipid content and a low value indicative of high fat content. For the liver/spleen ratio, there was no significant treatment (pioglitazone vs placebo) or time effect (0, 12, and 24 weeks) observed, nor a significant treatment by time interaction. However, in the pioglitazone-treated group there was a significant increase in the liver/spleen ratio, indicative of decreased liver fat, at 12 and 24 weeks ($P < .05$ at both times by paired t test) as opposed to the placebo group where there was no change (Table 3).

3.5. Visual analog scale estimation of hunger and satiety

By multivariate analysis, there was no effect of treatment ($P = .197$) on VAS questions 5 and 6. Question 5 was "How full does your stomach feel at this moment?" ($P = .01$). The average score of this question increased significantly in the pioglitazone-treated group ($P = .02$) and decreased, although not significantly, in the placebo-treated group. The score on question 6 ("How much food do you think you could eat at this moment?") increased significantly at 24 weeks in the placebo-treated group before the meal ($P = .02$), but there was no significant change in the pioglitazone-treated subjects ($P = .87$); at baseline and 24 weeks, the area under the curve over 6 hours after the thermal effect test meal was calculated and the difference analyzed with respect to the treatment. There was no significant pioglitazone effect on any of the questions.

3.6. Energy expenditure

There were no significant changes in RMR, postabsorptive respiratory quotient (RQ), or the TEF. Similarly, there were no significant changes in the fat or carbohydrate oxidation (COX) after a meal.

3.7. Discussion

This randomized, double-blind, placebo-controlled clinical trial compared pioglitazone and placebo treatment in diabetic patients who had not previously received treatment with TZDs. Pioglitazone improved average blood glucose as evidenced by a significant fall in HbA_{1c} in patients who were already in good glycemic control.

Weight gain was significantly greater in the pioglitazone-treated group within the first 4 weeks, and although it had increased by 3.88 kg, it had not reached a plateau by the end of the 6-month study. This is in harmony with several other studies that show weight gain after treatment with TZDs [10,11]. For example, King and Armstrong [18] showed a 5-kg weight gain that plateaued at 30 months. Given the low number of patients with fasting glycosuria (in each treatment group), it is unlikely that the change in body weight was due to reversal of glucose (energy) loss through the urine.

In the patients treated with pioglitazone, there was no significant change in lean body mass. Whole body fat, as measured by DEXA, increased by 3.55 kg in the pioglitazone-treated subjects. This value corresponds almost exactly to the increase in body weight (3.88 kg body weight vs 3.55 kg body fat). Also measured by DEXA, fat increased in the trunk, legs, and arms, indicating a generalized deposition of subcutaneous fat. A rise in body weight has also been observed in many other studies that have treated patients with TZDs [10,11]. It is possible that a fraction of the change in body weight is due to changes in total body water. It should be noted that CT scanning measures only lipid and also shows increased body fat. This increase in fat is to be expected from the mechanism of action for this drug. Thiazolidinediones are agonists for the PPAR-γ and initiate differentiation of adipocytes from preadipocytes into mature fat cells. Thus, treatment with this class of drugs would be expected to increase the number of small insulin-sensitive fat cells providing a reservoir for storage of fat.

During this 6-month trial, body fat increased by 3.88 kg or an increase of approximately 132 000 kJ (31 500 kcal). This amount of fat storage would require approximately 730 kJ/d (175 kcal/d) of positive energy balance. This is approximately 10% of the resting energy requirement for the people in this study (Table 5). We measured RMR, postabsorptive RQ, and the TEF, and substrate oxidation after a meal. Treatment with pioglitazone produced no significant changes in any of these measures of energy and fat/carbohydrate metabolism. There were small but nonsignificant trends in substrate oxidation toward fat storage and increased COX after a meal. This effect deserves additional study and consideration.

In the absence of data demonstrating an effect of pioglitazone treatment on resting energy expenditure or the TEF, one explanation for the change in body weight might be an increase in food intake. Although we did not measure food intake directly, most VAS measures of hunger and satiety, before and after a meal, did not change. The one VAS question for "fullness" that increased may represent an increase in abdominal fat, rather than an increase in satiety, per se. It is also possible that these measures are not sensitive enough to detect the estimated 730 kJ/d (175 kcal/d) energy excess required to change body weight by the observed gain of approximately 3.5 kg over 24 weeks. Because we did not perform measures of 24-hour energy expenditure and did not measure food intake directly, it is possible that changes in these parameters might account for the observed changes in body fat.

Multislice CT allowed us to calculate the volume of VAT [19,20], as opposed to the usual method of taking a single cross-sectional cut. With this technique, we found that the

Table 5
Baseline and changes in RMR, TEF, and RQ

Variable	Placebo		Pioglitazone		*P*		
	Baseline	24 wk	Baseline	24 wk	Treatment	Time	Interaction
RMR (kJ/24 h)	7004 ± 1475	196.5 ± 636	6978 ± 1390	−112 ± 804	.7037	.7729	.2575
TEF (% of intake)	7.75 ± 3.04	−0.35 ± 3.75	6.42 ± 3.54	0.13 ± 4.46	.1875	.9755	.7901
RQ (fasting)	0.87 ± 0.05	−0.008 ± 0.06	0.87 ± 0.04	0.007 ± 0.04	.9161	.9332	.4961
NPRQ	0.8922 ± 0.0807	−0.01 ± 0.08	0.8631 ± 0.0537	0.01 ± 0.05	.3874	.9356	.2355
COX (g/24 h)	123.24 ± 42.25	2.65 ± 58.90	119.79 ± 51.31	2.54 ± 39.08	.8631	.7731	.8810
FOX (g/24 h)	78.69 ± 62.70	10.68 ± 53.82	95.86 ± 42.04	−6.29 ± 48.48	.5635	.9031	.2712
POX (g/24 h)	80.34 ± 25.45	−4.96 ± 23.33	81.36 ± 35.61	−1.37 ± 23.32	.7618	.4119	.6801
Postmeal FOX	−4.24 ± 3.30	0.21 ± 4.59	−4.93 ± 2.94	−0.72 ± 4.80	.4830	.7036	.4839
Postmeal COX	6.87 ± 2.64	−0.18 ± 4.38	7.16 ± 2.72	1.09 ± 3.73	.2674	.4344	.2688

Data are mean ± SD. Nonprotein respiratory quotient (NPRQ) was measured 1 hour before the meal. Forty-two subjects (21 in each treatment group) were included in this analysis. Postmeal substrate oxidations are expressed as change from fasting values in grams per hour.

quantity of visceral fat did not change significantly during treatment with pioglitazone at the 24-week time point. Both prospective and retrospective power analyses demonstrate that we were well powered to detect changes in VAT. In the original power analysis, the SD of change in VAT (kg) was assumed to be 0.516 kg. In this study, we actually observed a smaller SD of change at 24 weeks of 0.469 kg. With 21 subjects per treatment group, there was more than 80% power to detect a difference of 0.4 kg (prior assumption). The actual observed difference in change from baseline between the two groups was 0.045 kg.

Several studies have shown an increase in subcutaneous fat during treatment with TZDs by using CT [6,7,21-23]. In 3 of these studies [7,22,23], there was a decrease in VAT, but not in another study [6]. The use of sulfonylureas in this latter study suggested that this might interfere with the response of VAT to TZDs. Miyazaki et al [24] showed a small but nonsignificant increase in VAT and SAT in a small number of Japanese patients. We tested this possibility by post hoc stratifying our patients into those who did and those who did not use sulfonylureas. The response to TZD did not differ by whether the patient received or did not receive sulfonylurea. Fullert et al [25] did not observe an increase in body weight in nondiabetic patients treated for 16 weeks with 45 mg/d of pioglitazone. Our observations suggest that the level of insulinemia is not correlated with weight gain in patients treated with pioglitazone weight (fat) change. In addition, the concomitant use of sulfonylurea was not associated with increased weight gain in subjects treated with pioglitazone. Taken together, the current data suggest that hyperinsulinemia, either due to insulin resistance or resulting from insulin secretagogues, is not a factor in the weight gain during TZD treatment.

Recent studies by Mayerson et al [26] demonstrated a decline in hepatic lipid content using a sensitive magnetic resonance spectroscopy technique. This was accompanied by an increase in the antilipolytic effect of insulin in SAT. In a similar set of experiments, Bajaj et al [11] demonstrated that the increase in body weight with TZD treatment is associated with a decrease in hepatic lipid content and a reduction in hepatic glucose production. Our results, measuring hepatic lipid content by CT scanning, are consistent with these data. One explanation for this finding is that fat shifts from "ectopic storage sites" such as the liver to SAT.

In summary, pioglitazone improved glucose control as evidenced by a fall in the hemoglobin A_{1c} and fasting glucose levels. Body weight and fat mass increased in subjects treated with pioglitazone, but VAT mass was essentially unchanged. Both DEXA and CT scanning revealed an increase in subcutaneous mass. This gain of body fat was apparent in the arms, legs, and trunk. This gain of body fat represents approximately 730 kJ/d (175 kcal/d) in energy storage and occurred in the absence of measurable changes in energy expenditure, RQ, the TEF, and postprandial substrate use. Subjective ratings of hunger/satiety as measured by VAS did not change with pioglitazone treatment. Neither fasting insulin values pretreatment nor the use of sulfonylureas at study entry was associated with the magnitude of weight change in patients treated with pioglitazone. In contrast to the untreated patient with diabetes, weight gain occurs without obvious deleterious effects on glucose control. Additional studies of 24-hour energy expenditure or more sensitive measures of food intake may be necessary to capture the subtle effects on physiological systems leading to the body fat gained while taking pioglitazone for diabetes control.

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